

Review

Dietary restriction in *C. elegans*: From rate-of-living effects to nutrient sensing pathways

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Abstract

The nematode *Caenorhabditis elegans* has been subjected to dietary restriction (DR) by a number of means, with varying results in terms of fecundity and lifespan. Two possible mechanisms by which DR increases lifespan are reduction of metabolic rate and reduction of insulin/IGF-1 signalling. Experimental tests have not supported either possibility. However, interaction studies suggest that DR and insulin/IGF-1 signalling may act in parallel on common regulated processes. In this review, we discuss recent developments in *C. elegans* DR research, including new discoveries about the biology of nutrient uptake in the gut, and the importance of invasion by the bacterial food source as a determinant of lifespan. The evidence that the effect of DR on lifespan in *C. elegans* is mediated by the TOR pathway is discussed. We conclude that the effect of DR on lifespan is likely to involve multiple mechanisms, which may differ according to the DR regimen used and the organism under study.

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1. What is dietary restriction?

Some 70 years ago, it was discovered that reduction of dietary intake without starvation substantially increases lifespan in rats (McCay et al., 1935). This treatment is referred to as dietary restriction (DR) or caloric restriction (CR). Since then similar effects have been observed in a wide range of species, from simple organisms such as baker's yeast *Saccharomyces cerevisiae* (Jiang et al., 2000) and the fruitfly *Drosophila* (Partridge et al., 1987; Chippindale et al., 1993; Chapman and Partridge, 1996), to a range of mammals, including mice (Weindruch et al., 1986; Bartke et al., 2001), dogs (Kealy et al., 2002) and possibly rhesus monkeys (Lane et al., 2002, 2004). Levels of DR which maximise longevity also result in reduced fertility (Holehan and Merry, 1986; Chippindale et al., 1993; Chapman and Partridge, 1996). It has been suggested that this response to DR represents an adaptation to increase fitness given

variable food availability. The hypothesis is that individuals may divert resources away from reproductive output, and into somatic maintenance during times of food scarcity. By this means, they may survive periods when it would be futile to attempt reproduction, thereby successfully postponing reproduction (Holliday, 1989; Masoro and Austad, 1996). The biological processes that determine longevity and ageing remain to be discovered; the effect of DR on lifespan provides a research tool to help identify these processes. In this review, we focus on one small corner of the large field of DR research: the study of DR in the nematode *C. elegans*. We cast a critical eye over the history of DR studies in this simple metazoan, and describe recent advances in the understanding of this topic.

2. Why study dietary restriction in *C. elegans*?

C. elegans has proved to be an excellent model for the study of the genetic determinants of longevity and ageing (reviewed in (Vanfleteren and Braeckman, 1999; Finch and

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Ruvkun, 2001)). A good example of this is the insulin/IGF-1 signalling pathway, whose role in ageing was discovered in *C. elegans* using classical genetic means, and then found to control longevity in fruitflies and rodents (Tatar et al., 2003). By contrast, the effects of DR on ageing were first discovered in a mammal. This being so, why investigate DR in a nematode? While DR effects on lifespan are widespread amongst animal species, there is no guarantee that the biological mechanisms involved are the same. Quite possibly, the effects of DR on ageing in different animal groups reflects convergent evolution, and involve different mechanisms in different taxa—or at least, a combination of mechanisms that are and are not evolutionarily conserved. Thus, there is a risk that studies of DR in *C. elegans* may lead to the discovery of at least some mechanisms of ageing that are nematode specific, and therefore, of more limited interest. Yet this potential disadvantage is counterbalanced by the great convenience of this organism as an experimental model (e.g., its 2–3-week lifespan; mice and rats live as many years), and the possibility of relating the biology of DR to the growing understanding of the broader biology of ageing in this organism.

3. How does digestion work in *C. elegans*?

Understanding the mechanisms of DR requires knowledge of the biology of nutrition. Geneticists working with *C. elegans* typically culture the animals on the surface of agar plates, in the presence of a lawn of the bacterium *Escherichia coli*, which is the food source. Frequently, a slow-growing auxotrophic strain of *E. coli* is used (OP50, uracil requiring); this results in a thinner bacterial lawn, making the nematodes easier to observe (Brenner, 1974).

In *C. elegans*, there are two major surfaces which act as interfaces with the nutrient environment. The first is at the chemosensory and olfactory neurones of the amphids; the second is the apical surface of intestinal cells. The gut is a good candidate for the site at which DR effects are exerted in *C. elegans*. Unfortunately, the biology of digestion represents something of a blind spot in this otherwise remarkably well-characterised organism. Before 2003, there was little information about how nutrients are absorbed from the gut lumen into the intestinal cells. A major text on the biology of the worm, *C. elegans* II, contains the surprising remark: “[The intestinal cells] may secrete digestive enzymes into the lumen” (Avery and Thomas, 1997). So little was known.

Early studies found that fine particulate matter in semi-defined media greatly improved nematode growth, suggesting that nutrient uptake occurs at least in part via endocytosis (Vanfleteren, 1974). Visual evidence for endocytosis came from studies feeding nematodes with fluorescently labelled probes (protein or dextran) of various sizes. Such probes accumulated within the autofluorescent gut granules, suggesting that fluid phase pinocytosis might be occurring

(Clokey and Jacobsen, 1986). Latex particles greater than 0.25 μM diameter were not taken up. For reference, an *E. coli* bacillus is $\sim 2 \mu\text{M}$ long and $\sim 1 \mu\text{M}$ thick. More recently, analysis of nutrient transport proteins has provided new insight into the biology of nutrient uptake in the worm (see Section 4). However, the biology of digestion and nutrient uptake remains largely obscure in this organism.

4. How to subject *C. elegans* to dietary restriction

Three approaches have been taken to subject *C. elegans* to DR: dilution of the bacterial food source, use of behavioural mutants which cannot feed normally, and culture in a semi-defined liquid medium in the absence of *E. coli*.

The first report of DR-induced longevity in *C. elegans* involved dilution of *E. coli* in buffer (S medium) (Klass, 1977). As expected, decreasing the *E. coli* concentration reduced fertility and increased lifespan. The *E. coli* concentration that maximised lifespan was 10 times lower than that which maximised fertility. A different approach used agar plate cultured nematodes, and reduced the thickness of bacterial lawns by reducing the nutrient content in the agar (Hosono et al., 1989). Again, as expected, an inverse relationship was seen between lifespan and bacterial quantity.

One goal of DR studies in *C. elegans* has been to relate the biology of DR to the various genes and pathways identified as determinants of ageing. Potentially, a convenient way to do this is to use DR mutants: strains where defects in feeding results in DR. Such DR-inducing mutations may then be combined with other mutations in a conventional epistasis analysis approach, *eat* mutations cause neuronal and muscular defects that impair pumping of the pharynx, resulting in slow-growth, and a starved appearance—the Eat phenotype (Avery, 1993; McKay et al., 2004). A number of Eat mutants were found to exhibit increased longevity; for example, *eat-1(ad427)*, *eat-2(ad1116)* and *eat-6(ad467)* were reported to increase lifespan by 33, 57 and 37%, respectively (Lakowski and Hekimi, 1998). However, it should be noted that in several other laboratories little or no increase in lifespan has been seen in *eat* mutants. For example, in the Gems laboratory, in multiple trials using several wild-type genetic backgrounds, only one of these three mutants (*eat-2*) showed an increase in lifespan, and this was of a smaller magnitude than reported by Lakowski and Hekimi (1998) (M. Keaney and D. Gems, unpublished). Other laboratories have confirmed the increased longevity of *eat-2* mutants (Hsu et al., 2003; Huang et al., 2004). *eat-2* was recently shown to encode a nicotinic acetylcholine receptor subunit (McKay et al., 2004).

The largest increases in *C. elegans* lifespan produced by altered nutrition are seen using a semi-defined sterile liquid medium (no *E. coli*)—so-called axenic medium (no other

species present) (Vanfleteren, 1980; Vanfleteren and Braeckman, 1999). The standard axenic medium consists of 3% soy peptone, 3% dry yeast extract, 0.5 mg/ml haemoglobin, and trace levels of sterols (which *C. elegans* cannot synthesise). Axenic culture typically results in a 50–80% increase in lifespan relative to monoxenic culture conditions, and this effect is highly reproducible.

Are the effects on lifespan of culture in axenic medium due to DR? This is not obvious, since axenic medium is rich in protein, carbohydrate, vitamins and minerals. Yet it also causes delayed development, and fecundity is much reduced. It seems likely that some characteristic of axenic medium leads to failure of nutrient uptake. One suggestion is that there is a failure of endocytic uptake of nutrients (Vanfleteren, 1974, 1980), although this has not been demonstrated experimentally. Other semi-defined and defined media have similar effects on development and lifespan (Vanfleteren, 1980).

Recently, understanding of nematode nutrient uptake has begun to open up thanks to several studies of intestinal nutrient transporter proteins. Mutant analysis of nutrient transporter genes has generated a further mode of DR-like intervention in *C. elegans*. Two types of nutrient uptake mechanism have been identified in this organism, involving the Na⁺-coupled transporters (NACs), homologues of the *drIndy* transporter, and the intestinal peptide (PEP) transporters, which are involved in the transport of tricarboxylic acid cycle (TCA) intermediates and dipeptides/tri-peptides, respectively. A Na⁺, H⁺ exchanger, NHX-2, modulates activity of the peptide transporter and so peptide uptake.

4.1. The sodium-coupled transporters

Mutation of *drIndy*, a dicarboxylate cotransporter of tricarboxylic acid cycle (TCA) intermediates, extends lifespan in *Drosophila* (Rogina et al., 2000). In *C. elegans*, three homologues of the *Drosophila Indy* have been identified: *nac-1*, *nac-2* and *nac-3* (Fei et al., 2003, 2004). The biochemistry of these three transporters has been well-characterised using heterologous expression systems, for example, in mammalian cell lines (Fei et al., 2003, 2004). The NACs are symporters; NAC-1 and NAC-3 are dicarboxylate transporters, while NAC-2 is a dicarboxylate/tricarboxylate transporter (Fei et al., 2003; Fei et al., 2004) (note that in *C. elegans* parlance, *nac-1* refers to the gene, and NAC-1 to the protein). In all cases, transport is Na⁺-dependent (unlike *drIndy*). In the case of NAC-2, it has been shown that transport is electrogenic, driven by an electrochemical potential gradient across the cell membrane (Fei et al., 2004). *nac-2* is the orthologue of both *drIndy* (despite the difference in Na⁺ dependence) and human NAC-2; the latter is found mainly in the liver, and is involved in the utilisation of extracellular citrate for hepatic lipid synthesis.

Interestingly, reduction of gene expression by RNA-mediated interference (RNAi) increases mean lifespan in the

case of *nac-2* and *nac-3* (but not *nac-1*) (Fei et al., 2003, 2004). In the case of *nac-2* this was +19%, and was accompanied by a ~40% decrease in body size and reduced lipid levels in the intestine (Fei et al., 2004). These other traits were not observed upon RNAi of *nac-3*, where a 15% increase in mean lifespan was seen (Fei et al., 2003). *nac-2* and *-3* both transport succinate, but *nac-2* transports citrate much more effectively (Fei et al., 2003, 2004). As well as being an intermediate in the TCA cycle, citrate is also a precursor for the synthesis of fatty acids, cholesterol and isoprenoids, and so may constitute a particularly important dietary component (Fei et al., 2004).

4.2. The *nhx-2* and *pep-2* transporters

PEP-2 (previously referred to as OPT-2) is found in the apical membrane of intestinal cells, and mediates the H⁺-dependent uptake of dipeptides and tripeptides from the gut lumen (Fei et al., 1998). Dipeptide/tripeptide uptake is therefore accompanied by H⁺ uptake, potentially resulting in acidification of the cytoplasm. This acidification is prevented by NHX-2 (Na⁺, H⁺ exchanger), which effects the electroneutral exchange of intracellular H⁺ with extracellular Na⁺ (Nehrke, 2003). Moreover, animals subjected to *nhx-2* RNAi exhibit a markedly slowed development and reach about half the maximal length of control adults. They also show reduced intestinal fat stores, slightly reduced pharyngeal pumping, near sterility, and increased lifespan (~40%) (Nehrke, 2003). Thus, these animals appear to be experiencing dietary restriction. *pep-2* (–) animals exhibit a similar but much weaker phenotype of reduced growth, body size and fertility, but no increase in lifespan (Nehrke, 2003; Meissner et al., 2004). This implies that disruption of *nhx-2* is affecting the activity of a number of nutrient transporters, including PEP-2.

5. How does DR extend lifespan in *C. elegans*?

The main topic of investigations on DR is the mechanism by which it extends lifespan, and this remains unclear. Numerous hypotheses have been put forward, and the literature on this topic is extensive, and has been reviewed elsewhere (e.g., Masoro, 2002). Two hypotheses that have been tested in *C. elegans* are that the effect of DR on lifespan stem from a reduction of metabolic rate, or of insulin/IGF-1 signalling.

5.1. The rate of living hypothesis

In both *C. elegans* and *Drosophila*, temperature strongly influences lifespan: the higher the temperature, the shorter the lifespan (Miquel et al., 1976; Klass, 1977). It has been suggested that such rate of living effects are attributable to the effects of respiration rate (Pearl, 1928). More recently, this rate of living theory has been linked to the oxidative

damage theory of ageing. According to this view, higher rates of metabolism result in increased production of damaging free radicals (e.g., superoxide and hydroxyl), increased molecular damage and reduced lifespan (Sohal and Weindruch, 1996; Beckman and Ames, 1998). However, the suggestion that the effects of DR on rodent lifespan are attributable to reduced metabolic rate has not generally been supported (Masoro et al., 1982; Masoro, 2000).

Rodent studies have strongly suggested that the lifespan-determining component of diet that is critical in the DR effect is the energy content (Weindruch and Walford, 1988), and for this reason in mammalian studies the term *caloric restriction* is frequently used. The importance of caloric intake in rodent DR might seem consistent with the idea that DR increases lifespan by reducing metabolic rate. However, in *C. elegans* as in *Drosophila* it remains unclear what components or properties of the diet are critical. For this reason, the use of the term caloric restriction in relation to the current *C. elegans* or *Drosophila* DR protocols is inappropriate.

Does DR increase lifespan in *C. elegans* by reducing metabolic rate? Lakowski and Hekimi (1998) observed that the combined effects on lifespan of mutations in *clk-1* and *eat* genes are less than additive, suggesting that these act via a common mechanism (Lakowski and Hekimi, 1998). As it was then thought that *clk-1* mutant longevity might result from reduced metabolic rate, this suggested that DR in the worm might act in a similar fashion. However, this was not supported by direct measurements of metabolic rate (either as oxygen consumption or heat production) in nematodes subjected to DR by bacterial dilution (Houthoofd et al., 2002a,b). *eat-2* mutant adults, in monoxenic liquid culture, were also examined; surprisingly, their metabolic rate was increased relative to wild type. A similar effect was seen in axenically cultured wild-type nematodes, relative to monoxenically-cultured controls (Houthoofd et al., 2002a). We may therefore conclude that in *C. elegans*, as in rodents, the effects of DR on lifespan are not attributable to reduced metabolic rate. Similar findings have recently been reported in *Drosophila* (Hulbert et al., 2004).

5.2. Insulin/IGF-1 signalling

It was discovered in *C. elegans* that reduction of insulin/IGF-1 signalling (IIS) could result in large increases in adult lifespan (Friedman and Johnson, 1988; Kenyon et al., 1993; Kimura et al., 1997). This finding was recently extended to fruitflies and rodents, demonstrating that the role of insulin/IGF-1 signalling in the control of lifespan is evolutionarily conserved (Partridge and Gems, 2002b; Tatar et al., 2003). One hypothesis is that DR increases lifespan by reducing IIS. In *C. elegans*, mutants with defects in chemosensation are long-lived, apparently due to reduced IIS, possibly reflecting a failure to detect food (Apfeld and Kenyon, 1999). Moreover, many of the *ins* genes, which encode putative insulins, are expressed in chemosensory neurones (Pierce et al., 2001).

These results suggest that the IIS pathway might mediate the response to DR. This would be an attractive hypothesis, since it could explain why the role of IIS in shortening lifespan evolved: to mediate trade-offs between fertility and lifespan in response to varying nutritional conditions (Partridge and Gems, 2002a). However, this hypothesis seems unlikely to be true, at least in *C. elegans*, for several reasons. Firstly, when one subjects IIS mutants to DR, a further extension of lifespan is seen. This is the case whether using bacterial dilution, *eat* mutants or axenic medium (Johnson et al., 1990; Vanfleteren and De Vreese, 1995; Lakowski and Hekimi, 1998; Houthoofd et al., 2003). Yet such additive interactions do not prove that DR and IIS act via different mechanisms. Each treatment alone could result in submaximal induction of the same longevity mechanism (Gems et al., 2002). In the case of the effect of axenic culture on IIS mutants, there is a synergistic interaction on mean lifespan (Vanfleteren and De Vreese, 1995; Houthoofd et al., 2003). This suggests that DR and IIS act in parallel on a common longevity-assurance process.

A more conclusive finding is that the effects of DR on lifespan do not require the DAF-16 FOXO transcription factor (Lakowski and Hekimi, 1998; Braeckman et al., 2000; Houthoofd et al., 2003). By contrast, the effect of IIS on lifespan is fully dependent on DAF-16 (Kenyon et al., 1993). This demonstrates that DR does not act on lifespan solely by down-regulating IIS. However, it is very possible that DR and DAF-16 act on common targets. In support of this idea, wild type *C. elegans* cultured in axenic medium were recently found to be stress resistant and to have elevated levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (Houthoofd et al., 2003), as seen in *C. elegans* mutants with reduced IIS (Larsen, 1993; Vanfleteren, 1993; Vanfleteren and De Vreese, 1995).

In *Drosophila*, dietary proteins and amino acids stimulate the IIS pathway (Zhang et al., 2000; Britton et al., 2002). There is some evidence of interactions between peptide uptake and IIS in *C. elegans*. Deletion of *pep-2* enhances the developmental arrest and longevity phenotypes of *daf-2* insulin/IGF-1 receptor mutants (Meissner et al., 2004). In *daf-2* mutants, mRNA levels of both *nhx-2* and *pep-2* are approximately eight-fold reduced, relative to *daf-16*; *daf-2* (McElwee et al., 2004). This suggests that IIS stimulates uptake of peptides (and perhaps other nutrients) by the intestine.

5.3. Sirtuins

Another potential mechanism of DR action is stimulation of NAD⁺-dependent histone deacetylases (sirtuins) (Blander and Guarente, 2004). This seems to be the case in *S. cerevisiae* (Lin et al., 2000), although see (Jiang et al., 2002; Kaerberlein et al., 2004), and *Drosophila* (Rogina and Helfand, 2004). In *C. elegans*, extra copies of *sir-2.1*, the orthologue of yeast Sir2, increases lifespan (Tissenbaum and Guarente, 2001). However, this effect requires *daf-16* which,

as we have seen, is in contrast to the effects of DR on lifespan in *C. elegans*. The possible role of *sir-2.1* in the response to DR in *C. elegans* remains to be tested directly. It has also been reported that the sirtuin-activating compound resveratrol increases *C. elegans* lifespan slightly (Wood et al., 2004).

6. Does DR in *C. elegans* act via Tor signalling?

As described above, the effects of DR on lifespan are not the result of reduced IIS or increased DAF-16 activity (Vanfleteren and De Vreese, 1995; Lakowski and Hekimi, 1998; Houthoofd et al., 2003). Yet the synergistic interaction between axenic culture and reduced IIS in their effects on lifespan (Vanfleteren and De Vreese, 1995) suggest that DR and reduced IIS act in parallel on common mechanisms which control lifespan. One candidate for a pathway mediating the effect of DR on lifespan is the target of rapamycin (TOR) pathway. The TOR kinase is part of a putative amino acid sensing pathway, which controls cell growth by altering gene transcription and translation (Oldham and Hafen, 2003; Hay and Sonenberg, 2004). When amino acid levels are high, TOR up-regulates translation through activation of ribosomal S6 kinase (S6K). When amino acid levels are low, reduced TOR signalling leads to up-regulation of autophagy: the degradation and turnover of proteins within lysosomal autophagosomes.

There is evidence of cross-talk between IIS and TOR signalling in *C. elegans*. IIS prevents formation of the developmentally arrested dauer larva (Riddle and Albert, 1997). If TOR acted with IIS, one would predict that reduced TOR activity would result in constitutive dauer larva formation. Mutation of the worm TOR gene (*let-363*) results in mid-larval arrest (Long et al., 2002; Jia et al., 2004). Initially, it was thought that this was not a dauer-like arrest (Long et al., 2002). However, after removal of a marker mutation linked to *let-363*, a dauer-like phenotype was revealed, similar to that resulting from mutation of *daf-15/raptor* (regulatory associated protein of TOR) (Jia et al., 2004). Moreover, RNAi of *let-363* increases adult lifespan, and this effect does not require *daf-16* (Vellai et al., 2003).

In *Drosophila*, reduction of TOR signalling increases lifespan, and it has been suggested that effects of DR on lifespan result from reduced TOR signalling (Kapahi et al., 2004). There is no direct evidence for a role of TOR signalling in DR in *C. elegans*. However, there is some indirect evidence. Deletion of *pep-2* enhances the *let-363* RNAi phenotype, suggesting that *pep-2* acts upstream of TOR-mediated nutrient sensing (Meissner et al., 2004). Synergy between axenic culture and reduced IIS could be explained in part by the down-regulation of *pep-2* and *nhx-2* with reduced IIS. Reduced IIS also leads to down-regulation of *daf-15*, as demonstrated by quantitative PCR (Jia et al., 2004). Taken together with the similar down-

regulation of *pep-2* and *nhx-2* (McElwee et al., 2004), this implies an integrated control of nutrient sensing and nutrient uptake by TOR and IIS (however, in the latter study, reduced transcription of *daf-15* with reduced IIS was not detected).

7. Is food toxic?

A long-standing concern about the effects of DR on lifespan in rodents is that under standard laboratory conditions they overeat, and this reduces their lifespan. If this were the case, DR might merely rescue this effect. An argument against this interpretation is that if DR merely rescued the harmful effects of overeating, one would expect it to increase fertility, not to decrease it (as is the case). The existence of distinct nutritional optima for fertility and lifespan is consistent with the evolutionary hypothesis that on low food, fertility is shut down to allow resource reallocation to longevity-assurance processes. This is a reasonable argument, yet not a compelling one. It remains logically possible that ageing is much more sensitive to toxic constituents of food than reproductive processes. An example of this is the effect of *E. coli* on lifespan and fertility in *C. elegans*.

Consider the condition of the elderly nematode, cultured on a lawn of live *E. coli*. One may easily imagine that frail, old nematodes might become vulnerable to toxins produced by the bacteria (Hansen et al., 1964), or to bacterial invasion. This possibility was tested by maintaining *C. elegans* throughout life on UV-killed *E. coli*. There resulted an increase in lifespan of ~20% relative to animals maintained on live *E. coli* (Gems and Riddle, 2000). Use of UV-killed *E. coli* did not slow down development or reduce fertility, implying that worms do not experience DR. These observations were confirmed and extended using antibiotics to prevent bacterial growth (Garigan et al., 2002). Moreover, it was observed that in elderly nematodes the mouth typically becomes blocked (“constipated”) with packed *E. coli* (Garigan et al., 2002). Thus, old, frail nematodes do indeed become vulnerable to invasion by their *E. coli* food source.

Clearly, one reason for the increased lifespan of axenically cultured *C. elegans* is the absence of these deleterious effects of *E. coli* (Hansen et al., 1964; Croll et al., 1977). However, nematodes raised on non-dividing *E. coli* are not nearly as long-lived as in axenic culture, and are fully fertile (unlike in axenic culture). Thus, freedom from the harmful effects of bacteria cannot fully account for the effects of axenic medium on life history (Mitchell et al., 1979). An untested possibility is that reduced bacterial invasion contributes to the life-extending effects of bacterial dilution.

That prevention of bacterial invasion is a mechanism by which at least one DR regimen can increase lifespan in *C. elegans* illustrates several important points. Firstly, we may

safely assume that bacterial invasion does not contribute to increased fertility early in life. Thus, this is an example of life extension via simple rescue of food toxicity—akin to the hypothetical rescue of the effects of chronic overeating in rodents referred to above. Secondly, it is clear that prevention of bacterial infection is only one way that *C. elegans* DR regimens can increase lifespan. Thus, there is not a single mechanism by which such regimens act in the worm. Thirdly, the effects of DR on rodent lifespan presumably do not involve rescue of the toxic effects of *E. coli*. Thus, this particular mechanism of action of DR in *C. elegans* is not evolutionarily conserved—it is *private* rather than *public* (Martin et al., 1996; Partridge and Gems, 2002b).

How might one go about testing the possibility that the life shortening effects of higher food levels are attributable to toxic effects on elderly nematodes or fruitflies? One possible approach, in principle, would be to adjust the levels of different food constituents in defined, isocaloric diets, on the assumption that some are more toxic than others. Unfortunately, in practice, this approach is not easy: defined diets per se result in DR-like effects in both organisms.

8. Ubiquinone restriction?

Another nutritional determinant of *C. elegans* lifespan is ubiquinone (coenzyme Q, or CoQ), which is an isoprenylated benzoquinone lipid important for electron transport in aerobic respiration. Long-lived *C. elegans* *clk-1* mutants exhibit profound CoQ auxotrophy, and require a dietary source of CoQ (Jonassen et al., 2001). Interestingly, maintenance of wild type *C. elegans* on *E. coli* mutants which cannot synthesize CoQ results in a substantial increase in lifespan (~60%) (Larsen et al., 2002); moreover, this effect, like that of other forms of *C. elegans* DR, does not require *daf-16*. Thus, CoQ is a dietary determinant of lifespan in *C. elegans* and CoQ deficiency represents a form of dietary restriction. The extent to which the effects on lifespan of bacterial dilution, *eat* mutations or axenic medium are attributable to deficiency of CoQ is unclear. However, it is noteworthy that *clk-1* mutants will not grow in axenic medium unless killed *E. coli* is added (Braeckman et al., 1999), i.e., axenic medium is deficient in CoQ. In the effects of CoQ we see an example of dietary restriction that is not due to caloric restriction.

9. Are DR mechanisms evolutionarily conserved?

Work on *C. elegans* currently supports the view that DR and IIS act in parallel to influence common mechanisms of longevity—assurance, mechanisms which include stress resistance, but not reduced metabolic rate. Is it likely that this pattern will, like the role of IIS in ageing, prove to be evolutionarily conserved? Potentially not. In *Drosophila*,

studies of the effect of a range of food concentrations on lifespan in the IIS pathway mutant *chico*¹ suggests that the DR could act by down-regulating IIS (Clancy et al., 2001).

The Ames dwarf mouse has combined pituitary hormone deficiency, and is long lived, possibly due to deficiencies in growth hormone and IGF-1 (Bartke, 2000). Subjecting Ames mice to DR resulted in a further increase in lifespan (Bartke et al., 2001). While this suggests distinct modes of action of DR and the Ames mutation, it does not rule out the opposite conclusion (see argument above) (Bartke et al., 2002). More recently, changes in gene expression in liver resulting from DR, the Ames mutations or both have been compared (Tsuchiya et al., 2004), and a striking overlap between the genes whose expression is altered was seen. This is reminiscent of the elevated antioxidant enzyme levels in *C. elegans* resulting from either DR or reduced IIS (Vanfleteren and De Vreese, 1995; Houthoofd et al., 2003). Moreover, additive effects of the Ames mutation and DR on expression were seen in many cases. This suggests that in both nematodes and mice, effects of DR and reduced IIS may act via common downstream mechanisms.

10. Conclusion: a paradigm shift in studies of DR?

To conclude, we suggest that there is a shift occurring in the understanding of DR studies in model organisms—a paradigm shift, if you like. The older, more straightforward view assumed that effects of diet on lifespan involved a single mechanism: reduced caloric intake. The influence of calories on lifespan was exerted via effects on metabolic rate, and production of reactive oxygen species (Krystal and Yu, 1994; Sohal and Weindruch, 1996). This was the expectation, whether the model organisms were rodents, fruitflies, nematodes or rotifers. This account of a view of DR action is summarised in Fig. 1. We certainly do not suggest that this reflects the view of all working in the field of DR; quite the contrary. Yet it was an influential view.

Old view: Unitary metabolic mechanism

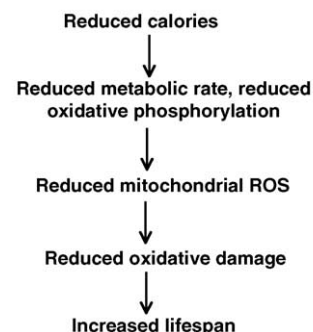


Fig. 1. Caloric restriction and the metabolic paradigm. This figure represents the way in which conceptions of DR action tended to be simplified rather than the way that many DR researchers have viewed DR during the last decades.

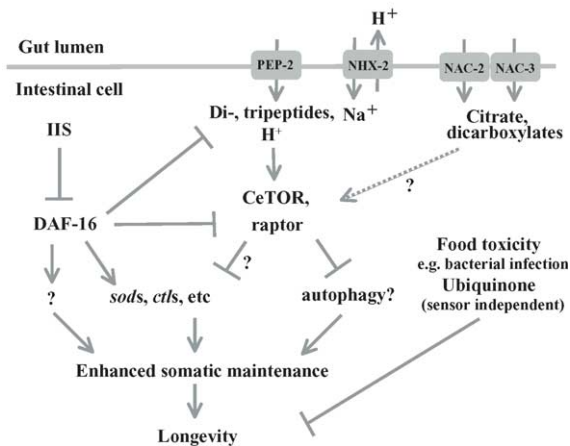
Complex view: Possible factors mediating DR in *C. elegans*

Fig. 2. Diet is likely to affect lifespan by more than one mechanism. This figure shows two types of mechanism: nutrient sensor mediated, regulated effects and toxicity effects, and toxicity and other effects. The TOR pathway is a candidate for a nutrient sensor system mediating the effects of DR in *C. elegans*. Although there is cross-talk between the insulin/IGF-1 and TOR pathways, the former does not mediate the effects of DR under the regimens tested; however, it can modulate these effects. Different nutrient sensor systems may act on ageing by the same or different effector mechanisms, or a mixture of the two. Two potential complications arising from DR studies are that (a) different DR regimens in a given organism may extend lifespan via different mechanisms and (b) different processes may mediate the effects of DR in different animal species.

This unitary view of DR has been succeeded by a more fragmented and complex view, summarised for *C. elegans* in Fig. 2. The possibility that the effect of DR on lifespan is attributable to reduced metabolic rate is not well supported by empirical studies (Houthoofd et al., 2002b; Masoro, 2002; Hulbert et al., 2004). Instead, a number of other, different mechanisms appear to be involved. These may include nutrient sensing systems, such as the interacting TOR and IIS systems in *Drosophila*. Thus far, no nutrient sensing system mediating the effects of DR has been identified in *C. elegans*. Moreover, the nature of the constituents or properties of the diet that determine the effects on ageing remains largely unknown for *C. elegans* or *Drosophila*. DR mechanisms may also include rescue of the toxic effects of the food source, as we have seen in the case of *E. coli* infection in *C. elegans*, or deficiency in dietary ubiquinone. An important question is the extent to which the mechanisms of DR are public versus private (i.e., evolutionarily conserved or not). Some mechanisms may be private: IIS does not mediate the effects of DR in *C. elegans*, but it might in *Drosophila*. Yet effects on SOD and catalase activity levels suggest that different pathways may converge on common longevity-assuring processes. A further potential complication is that in a given organism, DR administered in different ways may increase lifespan by different mechanisms. Perhaps a good warning to the DR investigator is: generalize with caution.

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